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Emerging technologies for metabolite generation and structural diversification

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ABSTRACT

Multiple technologies have emerged for structural diversification and efficient production of metabolites of drug molecules. These include expanded use of enzymatic and bioorganic transformations that mimic biological systems, biomimetic catalysis and electrochemical techniques. As this field continues to mature the breadth of transformations is growing beyond simple oxidative processes due in part to parallel development of more efficient catalytic methods for functionalization of unactivated scaffolds. These technologies allow for efficient structural diversification of both aromatic and aliphatic substrates in many cases via single step reactions without the use of protecting groups.

The generation of metabolites of active drug molecules is an important part of drug discovery as we attempt to understand the fate of pharmaceutical agents in the body. Typically, a circulating metabolite is sought out and isolated from an in vivo study as the result of poor PK or unexpected pharmacology. Confounding this analysis can be the transient or reactive nature of some metabolites.[1] Subsequent to tedious mass spectral analysis, a structure is proposed and a first attempt to prepare sufficient material might be carried out using liver slices or microsomes, for example.[2] When larger quantities are required, the task of producing a particular metabolite is assigned to the medicinal chemist via a dedicated synthetic route that may or may not intersect common building blocks. In addition this process occasionally requires redesign when the proposed molecule fails to match the isolated metabolite. Out of this need, various more efficient biomimetic technologies have been developed to aid medicinal chemistry teams in the production and study of metabolites.[2,3] The field of biomimetic chemistry has grown beyond simple oxidative transformations and now includes many other related disciplines that allow for structural diversification.[4] The purpose of this review is to highlight technologies related to structural diversification and generation of metabolites including enzymatic, catalytic and electrochemical methods. Together, these and other technologies are making single point structural modifications more efficient.[5]

The desire to selectively functionalize lead molecules is not new. For example, during the golden age of steroid research, teams utilized enzymatic transformations to create a diversity of biologically active steroids from plant based starting materials.[6] In the decades since, the understanding of the mechanisms underlying biological oxidation processes has increased[7] and research teams have been inspired to prepare novel metalloporphyrin catalysts that mimic these reactions. Research in this area continues to expand beyond metabolite production into the novel functionalization of common building blocks. However as Groves, et al. have pointed out, even with the recent advances in bioinorganic chemistry, the structure and reactivity of all of the metalloenzymes is not fully understood.[8] With the recent growth in the use of electrochemistry, the collection of efficient technologies available for structural diversification is beginning to grow. The ability to manipulate unfunctionalized molecules in ways that were previously inaccessible via traditional methods is particularly relevant

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to drug discovery for example.[9,10] Structural diversification allows the designer to navigate through safety and toxicity challenges while the ability to selectively oxidize molecules can be advantageous to physicochemical properties via lowering of the log *P*.

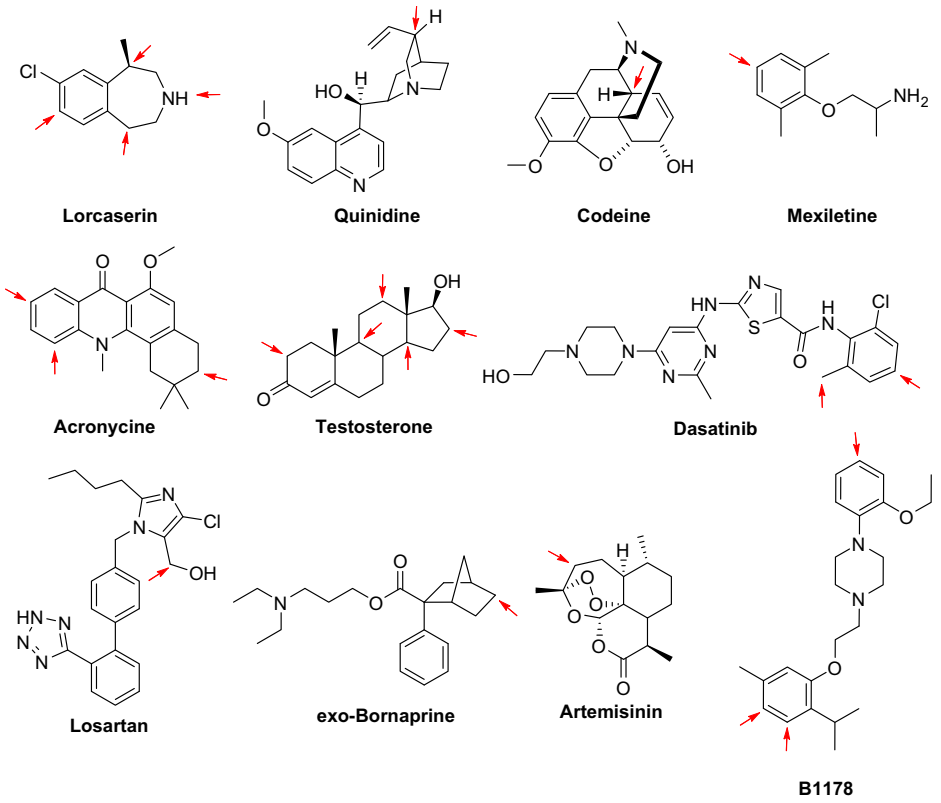
Biocatalysis: Access to specific drug metabolites is often key for the success of drug discovery programs and enzymatic transformations can contribute to the synthesis of metabolites and pseudometabolites (metabolites not observed in mammals). This section focuses on oxidative phase I metabolic transformations[11] involving cytochrome P_{450}s (CYPs), flavin-dependent monooxygenases (FMOs), monoamineoxidases (MAOs) and dehydrogenases.[12] Phase II transformations, while important, are beyond the scope of this digest.[13] Metabolic phase I transformations include a wide variety of reactions like dealkylations, epoxidations and isomerizations, but site-selective hydroxylations by direct C–H functionalization are among the most intriguing (Scheme 1).[14] The vast majority of hydroxylations in mammalian metabolism of xenobiotics results from the action of cytochrome P450s, in particular CYP1A2, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4.[15]

Besides microsomal preparations, pure recombinant CYP (rCYP) and FMO lyophilisates are standard tools with high value in metabolism-related R&D assays. However, compared to the use of recombinant enzymes, the expression of a certain enzyme in a bacterial host represents a modification of this technique being suitable for small scale preparative metabolite generation. Furthermore, the high potential of microorganisms in whole cells has been used for the creation of molecular diversity far beyond the observed mammalian metabolic transformations. One example being the construction of a CYP expression library based on *Escherichia coli* expression for P450 monooxygenases, which after careful screening and optimization revealed a rapid biotransformation-system on multi-well plates (Fig. 1).[16]

Microbial cultures provide higher enzyme activities, long-term stability and easier scale-up to prepare purified metabolites. These parameters support the use of whole cell enzymatic preparations as they have proven to be more efficient in terms of scalability of metabolite production, enzyme activity and costs. In addition, no regeneration system is required when using whole cell systems, offering another advantage.[17] Current practices within the pharmaceutical industry are trending towards the establishment of in-house screening technologies of representative diversity using both microbial biocatalysts as well as microsomal preparations and recombinant systems and the types of biocatalyst applications are summarized in Table 1.[18]

The direct correlation of bacterial or fungal P450s with specific human CYP isoforms or certain mammalian metabolite patterns still represents a future challenge but would tremendously facilitate strain selection. Remarkable progress has been made to optimize the correlation of microbial and mammalian oxidative drug metabolism during the last years by directed biocatalyst engineering.[19] Within the area of bacterial mutants, BM-3 variants of P450 derived from *Bacillus megaterium* (CYP 102A1) are of particular interest, as they accept a broader substrate range and offer greater potential for use at larger scale than human CYPs.[20] In this context, a drug library screening for metabolic activity towards a structurally diverse set of 43 drug-like compounds has been reported using BM3 mutants in cytosolic fractions.[21,22]

The application and combination of different methods such as human liver (HLM) and renal (HRM) microsomes, recombinant P450s and FMOs is illustrated by a recent report of Usmani et al. (Scheme 2).[23] The enzymes involved in the primary metabolism of Lorcaserin, a 5-HT_{2C} agonist, are described along with CYP inhibition experiments revealing the contribution of CYPs to the metabolic pathway.



Scheme 1. Diversity of enzymatic hydroxylation sites in various pharmacologically active compounds.[14]

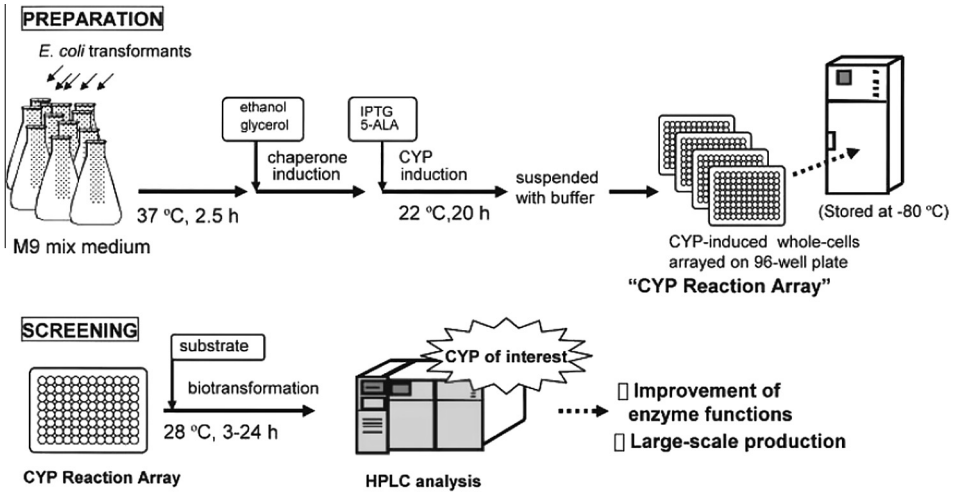
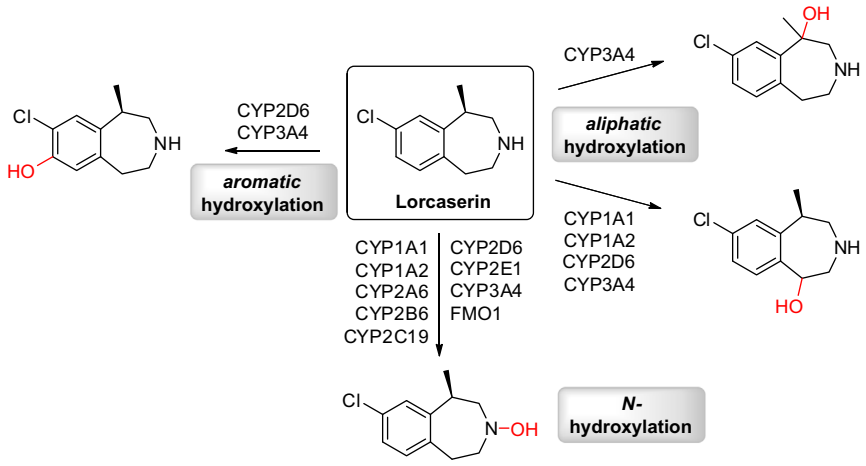


Figure 1. Preparation and use of a bacterial CYP reaction array. M9 mix medium: ampicillin medium to support robust and selective growth; Chaperone: protein assisting non-covalent folding/unfolding; IPTG: isopropyl β-D-1-thiogalactopyranoside; 5-ALA: δ-aminolevulinic acid.[16]

Table 1
Types of in vitro biocatalyst applications in metabolism[a]

Application	Suitable biocatalyst (enzyme preparation, whole cell, etc.)
Analytical profiling of drug candidates or drugs in identification of metabolic pathways or metabolic hot spots	Commercial (H)LM preparations rCYPs (human) as whole cells or microsomes [rCYPs (mammalian)] [Microbial biocatalysts,[b] whole cells]
Small-scale synthesis of human drug metabolites (milligram scale) for structure confirmation (NMR, MS)	rCYPs (human) as whole cells or microsomes [Microbial biocatalysts, whole cells]
Preparative scale synthesis of major human drug metabolites for PK and tox studies (10–100 mg) or derivatization (100 mg—multi-gram)	rCYPs (human) as whole cells or microsomes Microbial biocatalysts,[b] whole cells
Lead diversification: Identification and production of compounds with modified properties (100 mg—multi-gram)	Microbial biocatalysts,[b] whole cells

[a] Preferred methods given without brackets.[18]
[b] With known biocatalytic similarity to mammalian/human CYPs.

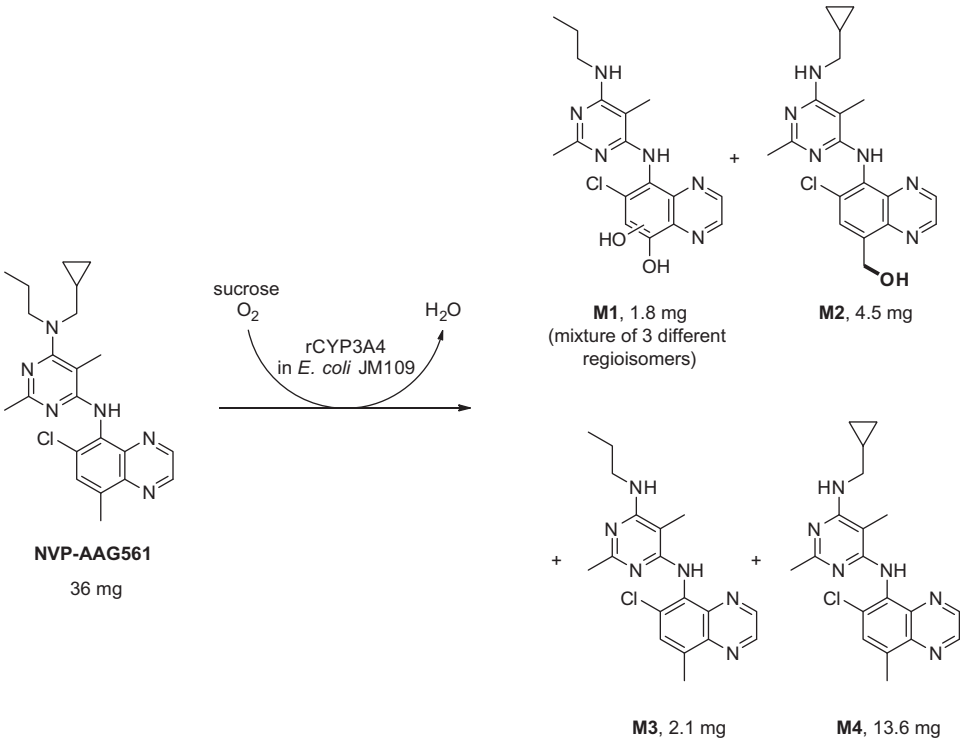


Scheme 2. Primary in vitro metabolites of lorcaserin produced by rCYPs.[65]

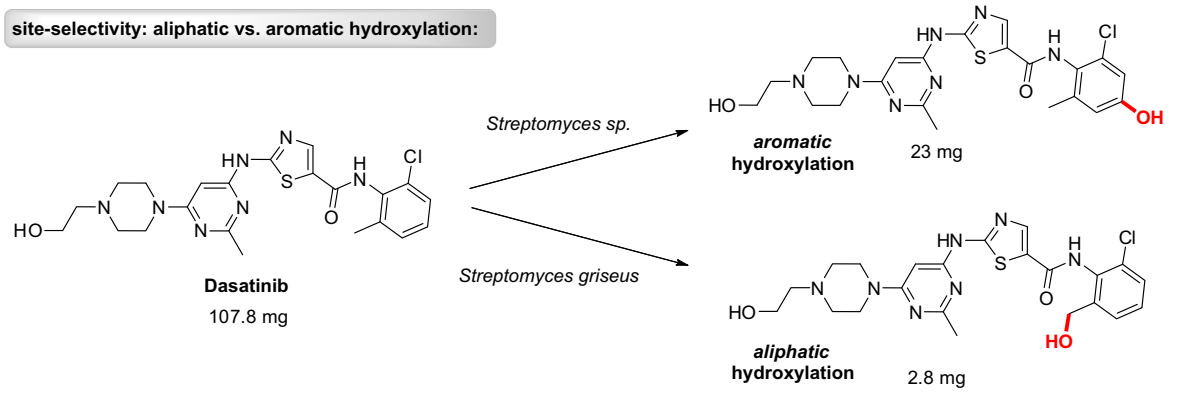
Advancing the rCYP methodology, the selective hydroxylation of the anti-depressant NVP-AAG561 by rCYP3A4 co-expressed in *E. coli* exemplifies the interface between recombinant systems and microbial transformations (Scheme 3).[18]

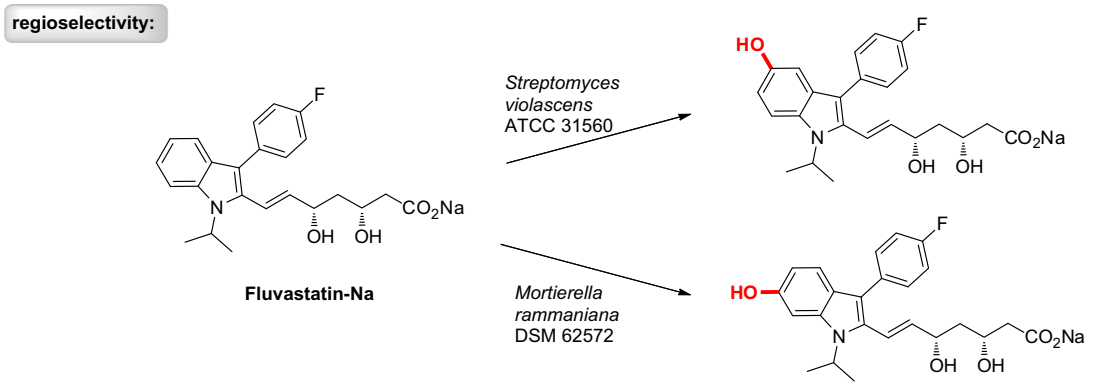
Aromatic versus aliphatic site-selectivity is attainable as in the case of the anti-cancer drug Dasatinib[24] while regiocontrol of aromatic hydroxylation is illustrated in the oxidation of Fluvastatin. Two very similar phenolic metabolites could successfully be synthesized with either shake-flask cultures or disposable bioreactor bags (Scheme 4).[25] Other examples include the synthesis of 100 mg quantities of the major active metabolites of Carbamazepine (Scheme 5).[26]

In addition, several contract research companies emerged over the last several years, offering screening and scale-up on a fee for



Scheme 3. Preparation of NVP-AAG561 metabolites with rCYP3A4 co-expressed in *Escherichia coli*.[18]





Scheme 4. Selective microbial hydroxylation: site- and regio-selectivity.[24,66,67]

service basis.[27] They also offer a panel of catalytic chemical reaction conditions using organometallic catalysts in a multi-well parallel format mimicking a synthetic liver[28] and CYP screening plates,[27] which have emerged as modern processes using different CYP, microbial or even fungal systems.

High lipophilicity of drug candidates leading to promiscuity, poor ADME and PK properties[29] is still one of the major challenges in drug discovery. Late-stage modification of active compounds and advanced intermediates has become an attractive approach to address these issues. Replacement of a hydrogen atom by a hydroxyl group significantly lowers lipophilicity and often leads to increased metabolic stability (Scheme 6).[30]

Enzymes with their high chemoselectivity and unique ability for direct C–H activation seem to be well suited for this type of late-stage aliphatic or aromatic oxidation in the presence of several other functional groups.

The lead optimization process is often influenced by results from metabolic studies driving attempts to either block metabolic hot spots or follow up on active metabolites with enhanced microsomal stability and improved pharmacokinetic properties. A classic example is the discovery of the cholesterol lowering agent Ezetimibe. In vivo studies of azetidinone derivatives like (−)-SCH 48461 led to reduction of serum cholesterol although the parent compound had hardly any acyl coenzyme A cholesterol acyltransferase inhibitory activity. Bioprofiling of the numerous metabolites of (−)-SCH 48461 revealed an active phenol metabolite. Subsequent optimization and blocking of a metabolic hot spot by fluorine eventually yielded Ezetimibe (Fig. 2).

A related approach describes the combination microbial and chemical methods for the late-stage fluorination of drug candidates to enhance microsomal stability.[31]

Complementary to developing screening sets of cytochrome P450 enzymes with activity for a wide variety of drug-like compounds, in silico drug metabolism tools have the potential to support the selection of cytochrome P450 subtypes for selective oxidation of drug candidates.[32] Cytochrome P450 mediated oxidations have impacted the design of prodrugs for a long time, for example, Tegafur is a prodrug for the thymidylate synthase inhibiting anticancer drug 5-fluorouracil that is not as rapidly degraded and is less toxic than the drug itself (Scheme 7). Future developments may include prodrugs targeting individual P450 enzymes to achieve organ and/or compartment specific release of the active drug. Particularly in cancer treatment more cytotoxic drugs could be delivered as prodrugs site-selectively to avoid systemic toxicity. Though not prohibitive common cytochrome P450-related problems like slow conversion of a prodrug and hence slow drug release, inter-patient variation and potential drug–drug interactions need to be considered.[33]

In the field of metabolite generation the traditional static enzymatic incubation more recently has been complemented by an advantageous flow-based approach: developments contain microfluidic hepatic co-culture platforms to enhance metabolite production and help to improve IVIV correlations[34] as well as lab-on-a-chip approaches imitating drug metabolism in PEGylated HLM, coupled with SPE purification and MS detection (Fig. 3).[35]

One of the latest developments in the use of cytochrome P450 BM3 mutants for structural diversification of compounds exploits the chiral environment of the iron(II)-porphyrin-containing binding pocket for asymmetric cyclopropanation of olefins with diazoacetic acid esters (Scheme 8).[36]

Wider use of enzymatic transformations for metabolite and/or pseudometabolite synthesis, as well as combinations of enzymatic with classic chemical reactions for late-stage derivatization of advanced intermediates and/or drug-like compounds are likely to find increased use in drug discovery in the future.

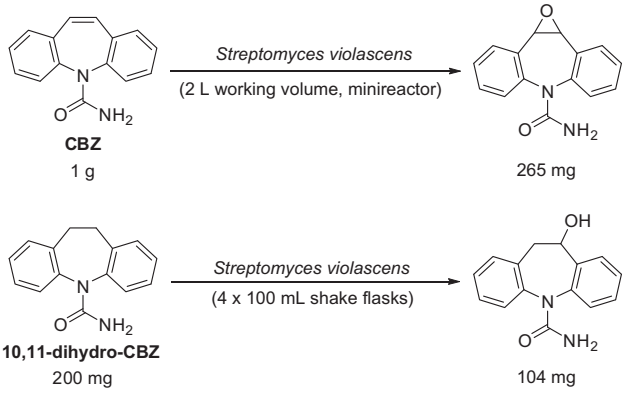
Biomimetic catalysis: Catalytic reactions continue to be a readily available source of transformations for bench scientists requiring little in the way of specialized equipment. New catalytic methods have been identified that greatly improve the step- and atom-economy of synthetic transformations thus transforming historically laborious processes into routine experiments. For example biomimetic metalloporphyrin catalysts and C–H activation protocols are now routinely utilized in the preparation of pseudometabolites and derivatives of the starting compound.[10] In addition, parallel development of catalysts for the direct fluorination of substrates provides an approach to minimize metabolism of drug molecules leading to fewer metabolites and potentially safer drugs.[37]

To date multiple model systems have been developed that mimic the transformations seen in vivo. The greatest value of these systems is the ability to produce multiple analogs in sufficient quantity to enable complete chemical characterization and pharmacological testing.[2] Metalloenzymes analogous to the panel of cytochrome P450 enzymes present in the human liver is commercially available for example.[28] The ability to utilize metalloporphyrin catalysts in vitro to prepare drug metabolites directly from parent drug is a significant leap forward for medicinal chemistry teams driving metabolite studies earlier into the drug development cycle. For example the discovery of both active metabolites of Nelfinavir could have been more systematic rather than serendipitous during human testing (Scheme 9).[38]

Similar advances in C–H insertion chemistry can be exploited to transform molecules in a single step via previously inefficient methods. The direct regioselective functionalization of aromatic and heteroaromatic compounds is now readily achievable and the reader is directed towards a recent review from Glorius et al.[10] for the scope of reactions in this field. Catalytic, nonporphyrin methods for C–H functionalization often provide an improved synthetic route to metabolites that mimics the selectivity of the metabolizing enzyme, but with significantly reduced reaction times and higher yields, thus allowing rapid access to key metabolites for further investigation and characterization. These methods provide a complementary approach in that the C–H bond that is oxidized can vary from the position that is selected by the enzyme. These alternative products provide novel analogs and new synthetic handles for further, late-stage compound functionalization.

M. Christina White and co-workers recently developed an electrophilic iron complex, Fe(PDP), which uses H_2O_2, an inexpensive, environmentally friendly oxidant to affect highly selective oxidations of tertiary sp^3 C–H bonds.[39] The reactions are run at ambient temperature and the catalyst achieves P450-like selectivity. An example of this reactivity is the oxidation of (+)-artemisinin (1) (Scheme 10), where the C-10 position is oxidized in 34% yield, 54% yield after recycling the starting material two times, to give (+)-10β-hydroxyartemisinin (2). Microbial cultures of *Cunninghamella echinulata* furnish a 47% yield of (+)-10β-hydroxyartemisinin (2) in 4 days.[40] Fe(*S*,*S*-PDP) (**3**) provides the product in higher overall yield than microbial cultures, in a shorter time (three 30 min reactions) and with a 10-fold higher volume throughput (0.033 M vs. 0.0035 M). A slow addition protocol can also be employed using the Fe catalyst, which removes the need for recycling starting materials to provide the product in 51% isolated yield.[41]

This method has been extended to include secondary aliphatic C–H bonds,[42–44] which are challenging to selectively oxidize due to their abundance in organic structures and their chemical inertness. The two-step oxidation of (−)-ambroxide (**4**) to (+)-2-oxo-sclareolide (**11**) demonstrates the selectivity of sequential oxidations with the first oxidation occurring at the C–H bond alpha to the ether to give (+)-(3*R*)-sclareolide (**5**) in 80% yield using 15 mol % of catalyst **7** (Scheme 11). The newly installed lactone now serves as an electron withdrawing group and deactivates



Scheme 5. Preparation of the anticonvulsive metabolites of the antiepileptic drug carbamazepine (CBZ).[26]

the B and C rings, resulting in oxidation of the C2 of the A ring to afford (+)-2-oxo-sclareolide (**11**) as the major product in 46% isolated yield employing 25 mol % of the Fe(*R*,*R*-PDP) catalyst (**7**). Microbial enzyme oxidations of (−)-ambroxide and (+)-sclareolide require longer reaction times (3–14 days) and provide a minimal yield of sclareolide (2%).[45,46] The major metabolite formed from fermentation of (−)-ambroxide with *Botrytis cinerea* is 1β-hydroxy-8-epiambrox (**6**). Importantly, no formation of oxo-sclareolide **11** or **12** was observed when sclareolide was treated with *Botrytis cinerea*, demonstrating the complementary nature of these catalysts to enzymatic oxidations.

The C–H functionalizations are guided by electronic, steric, and stereoelectronic effects. These effects can be overridden by the presence of a carboxylic acid in the molecule, which serves as a directing group.[48]

Costas and co-workers designed an iron-based catalyst with ligands similar to the ligand used by the White group but with pinene fused to the pyridine, with the hypothesis that the structurally elaborate ligand will provide both stability of the catalyst toward degradation pathways, allowing for lower catalyst loadings, and an increase in selectivity, closely mimicking an enzyme.[49] Changes in the regioselectivity of the oxidized products can be dictated by the chirality of the catalyst, the diamine ligand and the orientation of the pinene. Sclareolide was obtained in 70% yield utilizing only 3 mol % of catalyst **8** (Scheme 11). Subsequent oxidation of (+)-sclareolide occurs at the C1, C2, and C3 methylenes with varying selectivity. Changes in the selectivity ratios of the secondary C–H bonds that are oxidized, based on the catalyst that is employed, provide synthetically useful yields of products. Catalyst **13** provides an excellent yield (78%) of a mixture of **10**, **11**, and **12**. Interestingly, when catalyst **13** is utilized and the reaction temperature is lowered to −35 °C, oxidation at C1 yields **10** as the major product. Other catalysts are not active at this low temperature. This change in regioselectivity of the methylene oxidation demonstrates the orthogonal reactivity of this catalyst to others in the literature, providing access to different oxidation products.

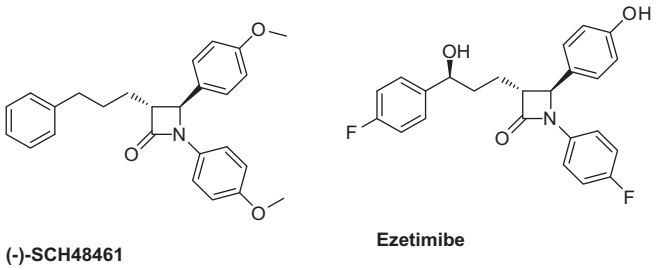
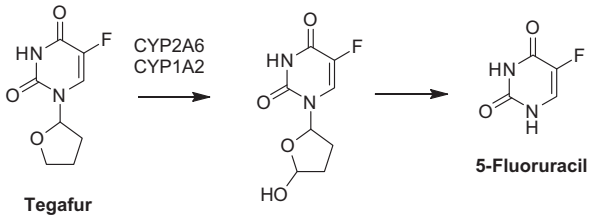


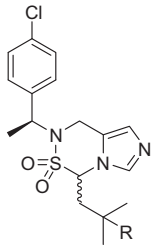
Figure 2. Lead compound (−)-SCH 48461 and its fluorinated analogue ezetimibe.

Aside from iron, other metals have also been explored for C–H oxidation (Table 2). A recent example is the Cp*Ir precatalysts by Crabtree and coworkers that use $NaIO_4$ as a mild oxidant.[50] The authors demonstrate the late-stage utility of this method by exploring oxidation of various natural products. No reaction occurred when artemisinin was subjected to **9**, which is in contrast to the iron complex developed by the White group that oxidized the C-10 methine vide supra. Sclareolide was obtained in 25% yield when (−)-ambroxide was subjected to 10 mol % of precatalyst **9** with oxidation occurring at the activated methylene, alpha to the oxygen (Scheme 11). Sclareolide oxidation using precatalyst **9** afforded 2-oxo-sclareolide (**11**) and 3-oxo-sclareolide (**12**) in 17% and 5% yield, respectively. Notably, this method and the others discussed above selectively oxidize unactivated methylene groups with numerous C–H bonds and a lactone present.

Nonheme catalysts should serve as powerful tools in drug discovery. These catalysts provide synthetically valuable yields of metabolites with significantly reduced reaction times. They also provide products that are complementary to those formed in enzymatic reactions. These alternative products provide both new molecules for characterization and also a functional group that can be exploited for further compound diversification. While recent examples for Phase I type oxidation have been discussed, other late-stage C–H functionalization reactions for compound diversification have also been developed.[51,52]



Scheme 7. Tegafur, a cytochrome P450-activated prodrug for the anticancer drug 5-fluorouracil.



R	clogD7.4	HLM t1/2 [min]	hCYP11B2 IC50 [nM]
H	3.28	6	9.4
OH	1.69	>300	11.4

Scheme 6. Hydroxylated aldosterone synthase inhibitor with enhanced microsomal stability and retained affinity.

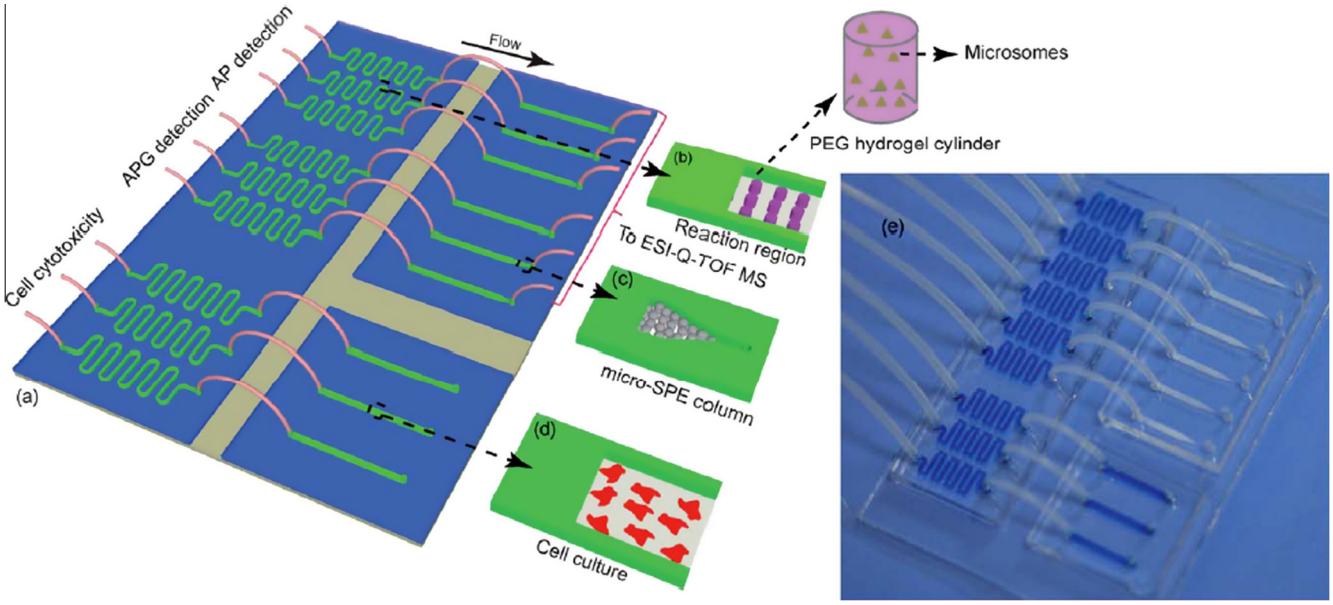
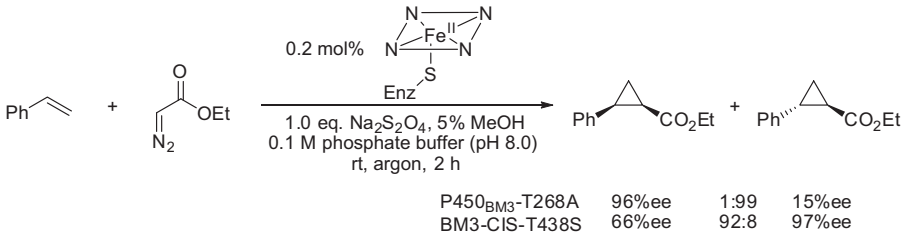


Figure 3. Microfluidic device for HLM metabolite analysis.[35] Microfluidic device for cell culture, metabolite analysis and cytotoxicity assay. (a) The integrated microfluidic device. (b) Microchannels for HLM encapsulation by PEG hydrogels. (c) Design of the on-chip micro-SPE column. (d) Cell culture channel. (e) An image of the microfluidic device filled with a blue dye in the bioreactor part cell culture part. AP: acetaminophen; APG: acetaminophen-glucuronides. Reproduced by permission of The Royal Society of Chemistry.



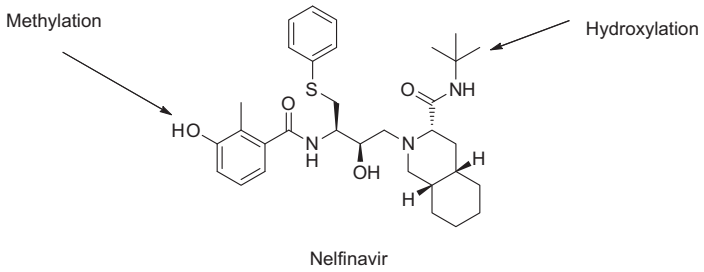
Scheme 8. Olefin cyclopropanation catalyzed by engineered cytochrome P450 enzymes.

Electrochemistry: Electrochemical methods have been proven to effectively yield different phase I reactions of drug molecules and are thus complimentary to both biotransformation and catalytic reactions. Reactions such as aromatic and benzylic hydroxylation, dehydrogenation, *O*-, and *N*-dealkylation, *S*-oxidation and less efficiently *N*-oxidation and *O*-dealkylation[53] as well as reaction with electrogenerated reactive oxygen species[54] have been demonstrated over the past years.

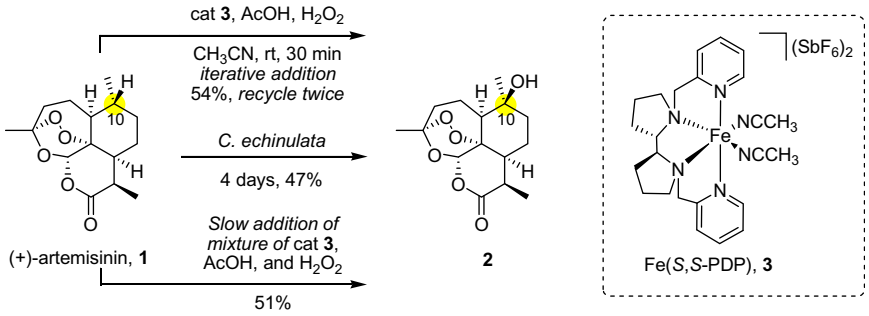
Different from other technologies, electrochemistry offers a purely instrument based approach without the need for isolation of metabolites out of complex biological mixtures and bears the potential for the rapid generation of larger amounts of metabolites and diversified molecules for subsequent testing. Furthermore, the generation of electrophilic metabolites in absence of biological nucleophiles provides access to unnaturally trapped intermediates that account for most severe side effects and allows directed mimicking of phase II metabolites.[55,56]

A drawback of electrochemical methods is that the position of lowest oxidation potential in a molecule does not necessarily mimic the observed sites in in vivo or other in vitro systems thus complementing the aforementioned technologies. The regioselectivity of a drug molecule oxidation is often more dependent of the topology of the active sites of the biological systems than of the effective oxidation energies within the drug molecule.

Although electrochemistry started with experiments in off-line batch reactors,[57] most contemporary systems for electrochemical metabolite generation feature either flow-based EC-MS or EC-LC–MS setups.[58] Another option is to incorporate the electrochemistry



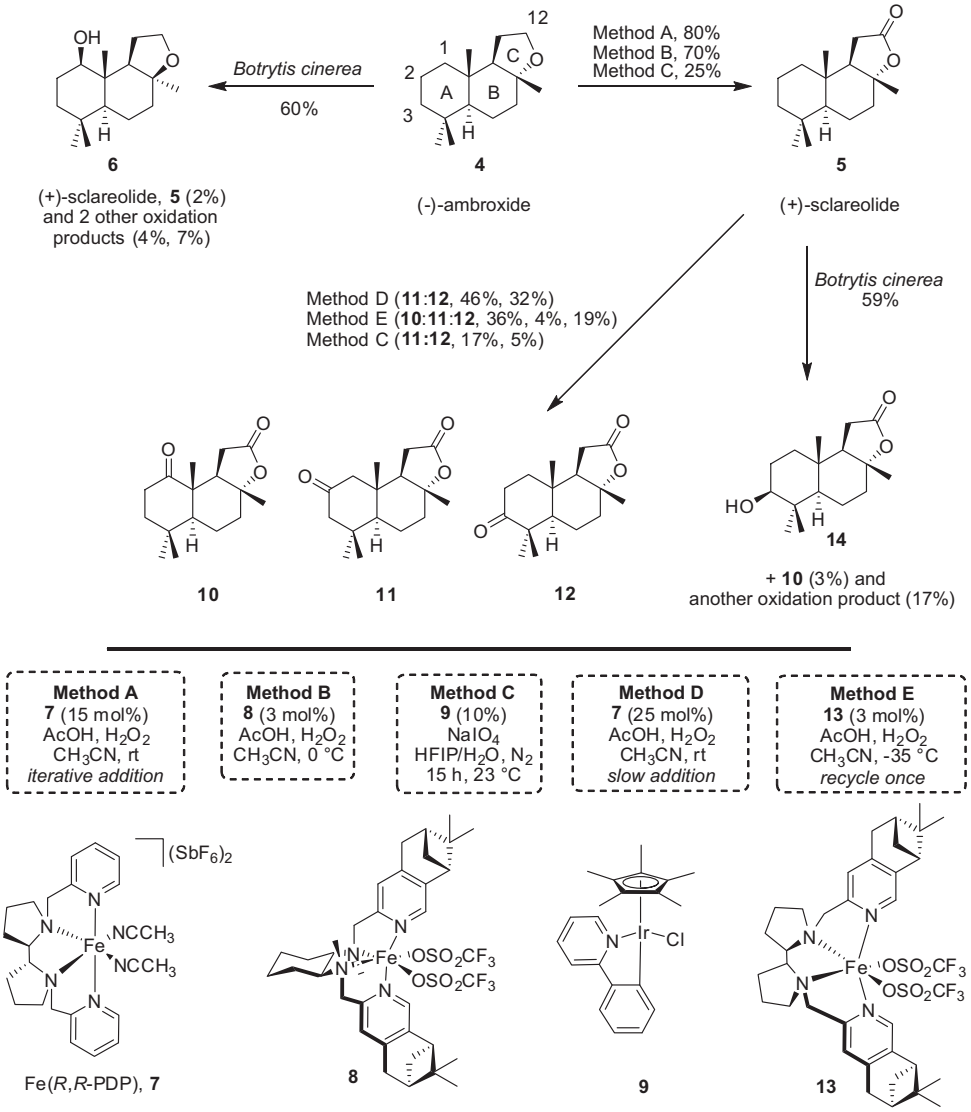
Scheme 9. Metabolism of nelfinavir producing active circulating metabolites.



Scheme 10. Oxidation of (+)-artemisinin. Reprinted with permission from Chemical Reviews. Copyright 2011 American Chemical Society.

within the electrospray source as the electrospray emitter induces electro-chemical reactions itself due to its high potential.[59]

Most current flow-through cells contain a three-electrode set-up, consisting of reference electrode, counter electrode and working electrode. These can be amperometric electrodes with exchangeable electrode materials in a thin layer cell setup or coulometric porous glassy carbon electrodes.[60] First microfluidic chips incorporating a thin-layer electrode geometry dedicated to drug metabolite generation were disclosed in 2009 by Odijk et al. demonstrating the Amodiaquine metabolite generation.[61] Later the group introduced an improved chip employing an iridium oxide based pseudo-reference electrode successfully mimicking the major metabolism pathways for procainamide.[62] Odijk and Qiao recently published a third generation microchip and demonstrated the oxidative metabolite formation of mitoxantrone.[63] Meanwhile, Kumacheva et al. reported a method for the fabrication of microflu-



Scheme 11. Oxidation of (−)-ambroxide and (+)-sclareolide.

idic electrochemical reactors based on soft lithography and micromolding in capillaries and demonstrated the synthetically interesting electrolyte-free anodic methoxylation of 2-pyrrolidinone to 5-methoxy-2-pyrrolidinone in methanol.[64]

Recent examples in the area of electrochemically generated metabolites show the increasing options of mimicking specific metabolism pathways (Table 3). For example, electrocatalytic oxidation of H_2O_2 on a platinum electrode generates reactive oxygen species, presumably surface-bound platinum-oxo species that are capable of oxygen insertion reactions in analogy to oxo-ferryl radical cations in the active site of Cytochrome P450 resulting only in hydroxylation in the 3- and 4-position of Lidocain (Table 3, entry 1).[65]

Permentier et al. extended the scope of electrochemical methods with the *O*-dealkylation of phenacetin to acetaminophen by square-wave potential pulses (Table 3, entry 6).[70] This reaction could not be achieved by oxidation at constant potential or longer potential pulses because of the fast hydrolysis of the reactive intermediates. By performing electrochemical reactions in non-aqueous systems Tahara et al. could generate and isolate reactive metabolites of Troglitazone that possibly account for the toxicity of the parent compound leading to its withdrawal from the market (Table 3, entry 7) thus proving electrochemical methods to be useful to prepare and predict reactive metabolites of drugs that are unstable in aqueous medium or in vivo.[71] The same group also reported one of the first semi-preparative scale syntheses of an in vivo metabolite by electrochemical methods for further NMR analysis.[72]

At this interface of analytical and preparative electrochemistry the growing field of meso-scale electrochemistry flow-cells[73] will also be of future interest for the generation of larger amounts of drug metabolites. This will not be limited to oxidative metabolites but also for late-stage modification by for example fluorination and trifluoromethylation reactions at particularly the positions of lowest oxidation potential. The underlying principle of most meso-scale cells is a plate-to-plate electrode configuration with distances in the micrometer order mounted in a non-conducting housing. Such systems have been employed in the synthesis of *p*-methoxybenzaldehyde dimethyl acetal out of *p*-methoxytoluene,[74] in the electrolyte-free anodic oxidation of furans in methanol[75] and also for acetoxylation of furan and benzene derivatives.[76]

Other examples include divided cells for the generation of *N*-acyliminium ions out of methyl pyrrolidinecarboxylate and their subsequent reaction with carbanions generated in a paired microflow system by cathodic reduction.[77] This chemistry and also the regioselective electrochemically-induced cross-coupling reaction of an aldehyde with allylic chloride were shown in a laminar flow-controlled microchannel without a membrane between the two compartments.[78] Yoshida et al. also introduced a carbon fiber electrode based microflow system in which the electric current

Table 2
Selected conversions and catalyst systems from recent literature examples[47]

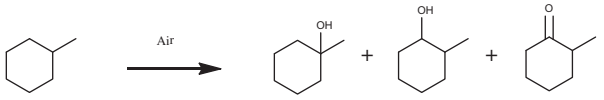
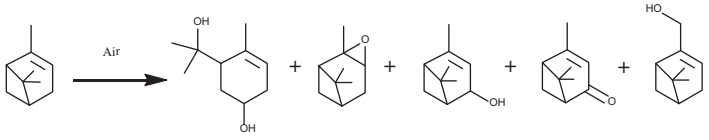
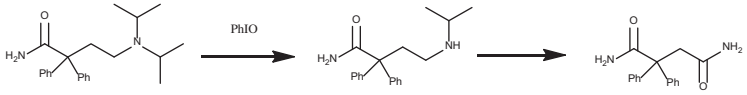
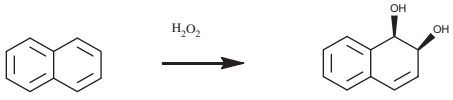
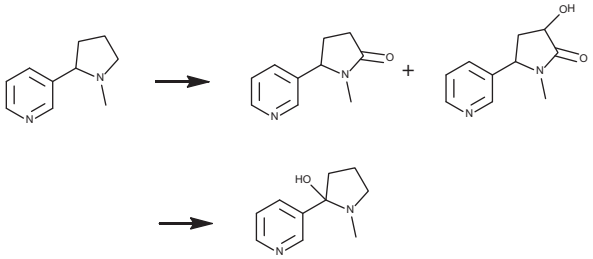
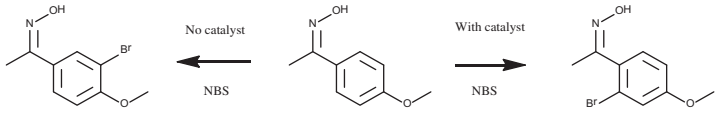
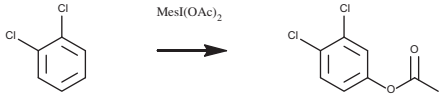
Entry	Reaction class	Metal	Representative transformation	Transformation	Refs
1	Biomimetic metalloporphyrin	Mn		Oxidation	47a
2	Biomimetic metalloporphyrin	Mn Co Fe Ni Cu Zn		Oxidation	47b
3	Biomimetic nonmetalloporphyrin	Fe		Oxidative degradation	47b
4	Biomimetic nonmetalloporphyrin	Fe		Oxidation	47d
5	Biomimetic metalloporphyrin	Fe Mn		Oxidation	47d
6	C–H Bond functionalization	Pd		Substrate directed fluorination	47e
7	C–H Bond functionalization	Pd		Electronically activated bromination	47e
8	C–H Bond functionalization	Pd		Catalyst activation	47e

Table 3
Selected metabolite structures and reaction systems from recent literature examples (BDD = boron doped diamond)

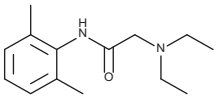
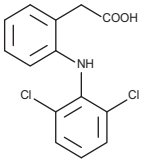
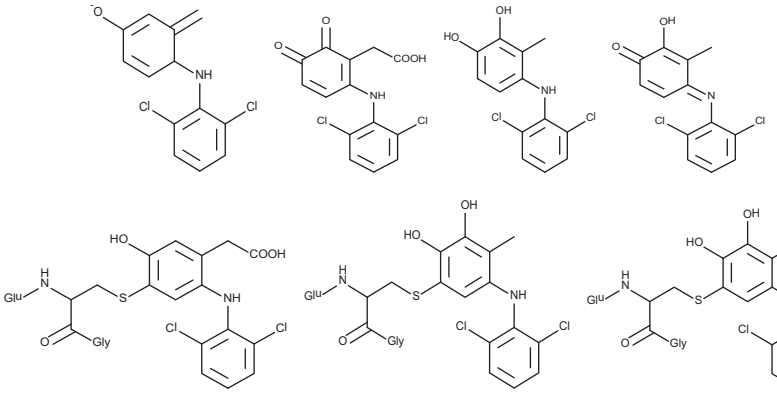
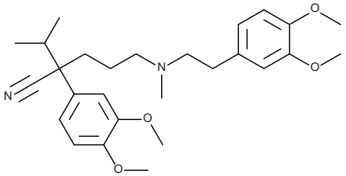
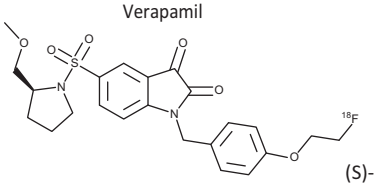
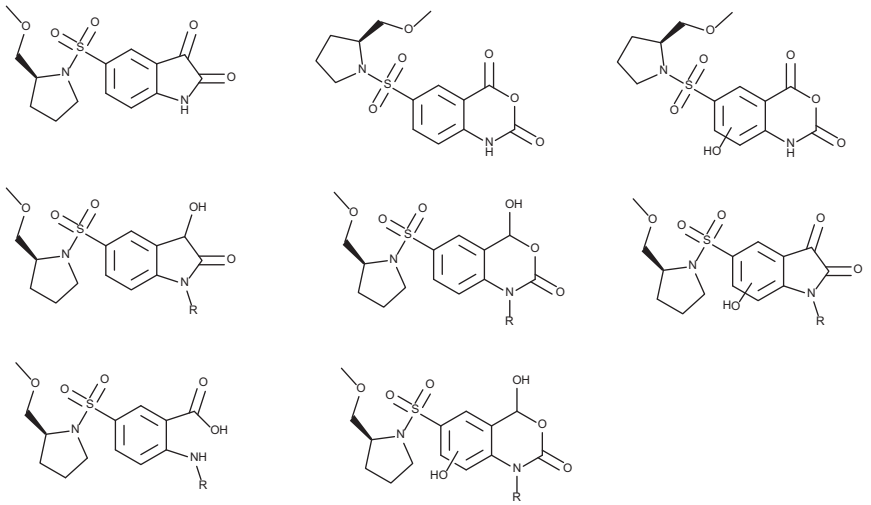
Entry	Drug	Type	Metabolites	System	Refs.
1	 Lidocain	P1	3-OH-Lidocain, 4-OH-Lidocain	Divided batch cell, H_2O_2, Pt electrodes	65
2	 Diclofenac (DCI)	P1, P2	5-OH-DCI, 4'-OH-DCI, 4-,5-diOH-DCI, 	Amperometric thin layer cell (EC/LC/MS), BDD electrode	66
3	 Verapamil	P1	All *in vivo* metabolites could be mimicked including *N*-dealkylated, *N*- or *O*-demethylated, oxygenated and dehydrogenated products plus additional non-naturally occurring derivatives	Amperometric thin layer cell (EC/LC/MS), BDD electrode	67
4	 (S)-1-(4-(2-[^{18}F]-fluoroethoxy)benzyl)-5-((2-(methoxymethyl)pyrrolidin-1-yl)sulfonyl)indoline-2,3-dione	P1	 R= 4-(2-[^{18}F]fluoroethoxy)benzyl	Amperometric thin layer cell (EC/LC/MS), Au electrode	68

Table 3 (*continued*)

Entry	Drug	Type	Metabolites	System	Refs.
5	Triclocarban	P1	All *in vivo* metabolites could be mimicked including mono- and dihydroxylated products plus additional non-naturally occurring derivatives.	Amperometric thin layer cell (EC/LC/MS), BDD electrode,	69
6	Phenacetin	P1		Divided batch cell, Pt electrodes	70
7	Troglitazone	P1 intermediate		Coulometric single-electrode cell, porous graphite electrode	71

flow and the liquid flow are parallel. Employing a porous PTFE spacer membrane the anodic methoxylation of *p*-methoxytoluene was conducted in an electrolyte-free reaction.[79] A C–C bond formation reaction was reported by Haswell et al. in the electro-reductive coupling of activated olefins and benzyl bromide between platinum electrodes.[80]

By adapting strategies from metabolite generation those mesoscale cells will in the future help to generate preparative amounts of highly desirable late-stage diversified drug molecules that are otherwise tedious to synthesize.

In summary, an integral part of the drug discovery and development process involves characterization of the metabolites of a drug candidate. At the lead optimization stage, metabolite identification can aid drug design efforts on several fronts. The most important one is the identification of metabolically labile positions for compounds with high clearance. In addition, this helps determine whether any of the pharmacological activity of the drug is due to active metabolites. This has gained further importance due to the recent FDA guidance on the importance of characterizing metabolites at a pre-clinical stage.[81]

The ability to directly access functionalized molecules more efficiently than conventional synthetic chemistry techniques is very attractive to medicinal chemists.[82] From a drug discovery perspective, the ability to introduce hydroxy groups in drug candidates is a powerful technique to modulate physicochemical properties late in the lead optimization process. Furthermore, new efficient methods have emerged, enabling regioselective fluorination of aromatic substrates to improve metabolic stability.[37]

This perspective has aimed to highlight three complementary areas that are available to the medicinal chemists, tasked with the multi-parametric issues of iterative compound design and optimization. Enzymatic transformations represent perhaps the most well-studied and developed of the three approaches described in this perspective. Many fungal and bacteria strains with the capacity for oxidative biotransformation, are useful for the biosynthesis of otherwise difficult to prepare compounds. As mentioned in the introduction, much of the knowledge about these microbial techniques comes from screening efforts to identify strains that metabolize steroid-like molecules. Evidence indicates that many fungi are capable of cytochrome P450 super family-like oxidative biotransformations. Although cultures of these fungi may provide a convenient and abundant capacity for enzymatic oxidations, fermentations in a micro titer plate or a shake flask can be difficult to manipulate in terms of throughput and isolation. As such, the availability of a screening kit of BM3 P450 mutants represents a convenient entry to those interested in investigating the substrate diversity and product profile of this class of enzymes. Further, the substrate scope and selectivity profile attainable with rCYPs, as highlighted by the functionalization of Dasatinib and Fluvastatin represents a powerful example of this technique. The chemoselectivity and unique ability for direct C–H activation also expands the scope towards lead diversification or preparation of non-human metabolites, as illustrated in Scheme 4. Given industry-wide efforts to drive towards compounds with lower *c*Log*P*,[83] this avenue will continue to aid drug discovery scientists. Porphyrin-based catalytic methods offer a powerful approach to enable selective functionalization of unactivated C–H bonds in a similar manner to cytochrome P450-based metabolism pathways. Interest in this field is expected to grow, with recent examples of porphyrin-mediated chlorinations being an attractive example of the potential expansion in generality and scope. Among nonporphyrin based methods, the development of Fe and Cp*Ir precatalysts represent emerging areas for late-stage functionalization of complex molecules. Important in the utility to practicing medicinal chemists is the ease of experimental setup to screen multiple catalyst systems, followed by state of the art analytical and separation tools to enable rapid decisions. Complementary to both these tech-

niques are electrochemistry-based approaches for structural diversification. While this technique can generate metabolites mimicking specific metabolism pathways as shown in Table 3, the potential to 'trap' reactive metabolites and functionalize them with a range of nucleophiles, both endogenous and others, offers access to a wide array of metabolites and diverse structures. In addition, recent advances in meso-scale electrochemistry enabling the generation of larger amounts of desired compounds will prove to be quite attractive for medicinal chemists.

While this perspective is not intended to be a comprehensive review of the significant amount of research that has, and continues to occur in these three areas, representative examples of the scope and complementarity of available tools are highlighted. Continued refinement, in terms of predictability and ease of use, will be important to drive these techniques into mainstream medicinal chemistry efforts. Extension of the utility of each of these technologies beyond simple oxidative transformation is known[9,84,85] and the potential of these tools is enormous. These methods coupled with predictive tools[84a] are speeding the production and analysis of metabolites and enabling earlier profiling within the drug discovery funnel. As innovative new transformations are identified, the toolbox available to the synthetic chemist will continue to grow.

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